



Multivariate optimisation of ICP OES instrumental parameters for Pb/Ba/Sb measurement in gunshot residues

Gabriela Vanini ^{a,*}, Murilo O. Souza ^a, Maria T.W.D. Carneiro ^a, Paulo R. Filgueiras ^a, Roy E. Bruns ^b, Wanderson Romão ^{a,c,*}

^a *Petroleomics and Forensic Laboratory, Department of Chemistry, Federal University of Espírito Santo (Universidade Federal do Espírito Santo – UFES), 29075-910 Vitória, Espírito Santo (ES), Brazil*

^b *Institute of Chemistry, State University of Campinas (Universidade Estadual de Campinas – UNICAMP), Cidade Universitária Zeferino Vaz, 13083-970 Campinas, São Paulo (SP), Brazil*

^c *Federal Institute of Espírito Santo (Instituto Federal do Espírito Santo – IFES), 29106-010 Vila Velha, Espírito Santo (ES), Brazil*

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ABSTRACT

Inductively coupled plasma-optical emission spectrometry (ICP OES) is a powerful analytical tool for the determination of metals. It is simple, fast and inexpensive due to its high sensitivity and versatility, and it can be used to analyse multiple elements. However, its use in forensic chemistry remains underexplored. This study aims to optimise the analysis of gunshot residues (GSRs) by ICP OES using the concentrations of Pb, Ba and Sb released during the firing of ammunition. In this study, experiments were designed using multivariate optimisation to establish the best operating conditions for ICP OES in GSR samples generated from a .38 revolver. Three variables were studied (radio frequency power, nebuliser gas flow and aspiration rate), and the interactions between those variables were investigated using a central composite design to establish the best analysis parameters. The nebuliser gas flow was the most important parameter in the optimization of the signal intensities, and the interactions between the variables were not significant. The optimum values determined for radio frequency power, nebuliser gas flow and aspiration rate were 1300 W, 1.2 L min⁻¹, and 1.0 mL min⁻¹, respectively. After optimisation of the parameters, the performance characteristics of the proposed method were established: linearity, detection and quantification limits, and accuracy (recovery percentage), with and without addition of an internal standard.

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1. Introduction

The use of analytical techniques to identify gunshot residues (GSRs) in forensic analysis has largely replaced traditional qualitative methods such as wet assays (the Feigl–Suter reaction) [1–4]. The inductively coupled plasma-optical emission spectrometry (ICP OES) technique [5,6] is promising in this regard because it has good sensitivity for the determination of metals, in addition to being multi-element, simple, fast and inexpensive compared to other techniques, such as scanning electron microscopy–energy dispersive X-ray (SEM/EDX) detection [7–10] and inductively coupled plasma-mass spectrometry (ICP-MS) [11–13].

The elements Pb, Ba and Sb are the main chemical markers present in the inorganic residues released after a gunshot, and forensic

laboratories routinely seek to identify these metals [14]. The use of the ICP OES technique for GSR analysis is infrequently reported in the literature [5,6,12]. An in-depth study to establish the ideal operating conditions is crucial for obtaining reliable results.

For ICP OES, various instrumental parameters must be initially established: radio frequency (RF) power, torch configuration (axial or radial), plasma, nebuliser and auxiliary (coolant) gas flow and aspiration rate. Statistics are used in this context because they aid the resolution of problems involving several variables that are affected by a large number of factors. Thus, using an experimental design based on statistical principles and associated with the technical and scientific knowledge of the researcher, the system can be optimised using a minimum number of experiments [15].

Multivariate optimisation has been widely used to establish the optimal operating conditions of analytical techniques [16,17] by selecting the best analysis parameters from the interactions existing among them [18]. The design method to be used depends on the objective proposed for the experiment, and the interaction between variables can only be evaluated using multivariate methods, as univariate methods evaluate the effect of one variable at a time, keeping all other variables constant.

* Corresponding authors at: Av. Fernando Ferrari, 514, Goiabeiras, Núcleo de Competências em Química do Petróleo, Vitória, Espírito Santo (ES) 29075-910, Brazil. Tel.: +55 27 3145 4502.

E-mail addresses: gabrielavanini@hotmail.com (G. Vanini), wandersonromao@gmail.com (W. Romão).

Lyra et al. [19] used two design methods (univariate and multivariate) in their study of phosphorus detection in biodiesel using graphite furnace-atomic absorption spectrometry (GF-AAS). The pyrolysis, atomisation temperatures and chemical modifier mass were obtained by a multivariate method, and the other steps of the temperature programme were obtained using univariate analysis. In ICP OES analyses, multivariate optimisation has been widely reported. Chirinos et al. [20] measured aluminium, copper, magnesium, chromium and iron by ICP OES using a 2^3 factorial design for the parameters RF power, nebuliser pressure and auxiliary gas flow rate. Trevisan et al. [21] used an experimental design to assess the ICP OES plasma conditions in relation to axial and radial views for two liquid samples, and the variables studied were RF power and nebuliser gas flow rate. Froes et al. [22] also used a 2^3 factorial design to assess the RF power, nebuliser gas flow rate and aspiration rate parameters in ICP OES for the identification of metals in fruit juices.

In the present study, three variables were selected and studied: RF power, nebuliser gas flow rate and aspiration rate. These parameters were chosen because they are very important in ICP OES analysis, based on previous optimisation studies [19–22]. For the optimisation of these factors, the central composite design (CCD) was used for the three factors. This design consists of three parts [15] and is indicated for long experiments:

- 1st part Factorial or cubic step, containing a total of $n_{\text{fact}} = 2^3 = 8$ coordinate points $x_i = -1$ or $x_i = +1$, for all $i = 1, 2, 3$;
- 2nd part Axial or star step, formed by $n_{\text{ax}} = 2 \times 3$ points with all null coordinates, except one, which is equal to value α (or $-\alpha$). In this case, $\alpha = (\text{number of factors})^{1/4} = 1.68$.
- 3rd part A total of n_{centr} assays performed in the central point, where $x_1 = x_2 = x_3 = 0$.

The CCD is also called a spherical design because the cubic and axial points lie on a roughly spherical surface, justifying the value of α [15]. Other key features include the fact that replicates occur at the central point, and in this study, triplicate tests were performed to provide a measure of pure error and to establish the variance of the predicted response [15]. The CCD is an adequate design to determine linear and quadratic models to represent the experimental data.

The reliability of the model obtained by the CCD can be verified using ANOVA (analysis of variance), which determines the variance components of the experimental data. The total variance of the model is decomposed into two parts, which are regression- and residual-related. These components are used to test the significance of the regression using the F distribution. The variance of residuals can be decomposed into two parts, one due to pure error (determined by the variance of the replicate at the central point) and one due to model imperfections, called lack of fit. An F test can be conducted with these two variances, and their degrees of freedom can be used to determine the degree of fit of the model to the experimental data. In this study, the development of an ICP OES optimisation method to measure the metals Pb, Ba and Sb in GSR using CCD multivariate optimisation was discussed.

2. Experimental methods

2.1. Instrumentation

An ICP OES instrument (Perkin Elmer, Optima 7000, USA) was used for the quantification of Pb, Ba and Sb. The instrument contained a Meinhard concentric pneumatic nebuliser and a cyclonic nebuliser chamber attached to a peristaltic pump. Both are used to introduce the samples into the plasma. An axial view and the following spectral lines were used: Pb 220.353 nm, Ba 233.527 nm and Sb 206.826 nm. For the preparation step, an ultrasonic bath was used (UltraCleaner 1450, Unique, Brazil).

2.2. Materials and reagents

Ultrapure 65% nitric acid (HNO_3) (Merck, Germany), ultrapure water (1 + type, with resistivity = $18.2 \text{ M}\Omega \cdot \text{cm}$) prepared by a reverse osmosis system (Purelab Ultra Mk2, United Kingdom), hydrogen peroxide, H_2O_2 (Cromoline Química Fina, Brazil) and ethylenediaminetetraacetic acid (EDTA) (Sigma-Aldrich, USA) were used for preparation and extraction of the samples. EDTA was used as a complexing agent on moistened swabs. All reagents and solvents were used as received. A multi-element stock solution ($1000 \mu\text{g L}^{-1}$) of the Pb, Ba and Sb standards (Sigma Aldrich, Switzerland) was prepared to build a calibration curve ($100, 200, 300$ and $500 \mu\text{g} \cdot \text{L}^{-1}$). All standard solutions were acidified with 2% HNO_3 (v/v).

2.3. Collection and preparation of samples

The GSR samples were collected at the Laboratory of Ballistics of the Institute of Criminology in the city of Vitória, Espírito Santo state, Brazil. The GSRs were collected from a single shooter, who fired three shots in duplicate. For this step, a .38 Taurus® RT 85 revolver and complete CBC 38SPL Lead cartridges were used. Swabs soaked in a 2% EDTA solution (w/v) were used for the smear technique. The time allotted for each sample collection (swab with the hand) was 1 min.

The swabs containing GSR were stored in 15 mL polypropylene tubes. A 2 mL volume of a 10% HNO_3 solution (v/v) was added to each tube, and the samples were diluted to a final volume of 10 mL. The samples were then subjected to an ultrasonic bath for 20 min at 25 kHz and heated in a water bath for 1 h at 100°C . Finally, the swabs were removed from the tubes, and the resulting solutions were analysed by ICP OES.

2.4. Optimisation of operating conditions

The parameters optimised to maximise the ICP OES sensitivity were RF power, nebuliser gas flow and aspiration rate by applying CCD. All experiments were performed with GSR samples, and the analytical response was the emission intensities of each metal. A total of 17 assays ($2^K + 2K + 3$, where K is the number of study variables, in this case $2^3 + 2 \times 3 + 3 = 17$) were performed, of which three were replicates of the central point used to calculate the standard error. The entire optimisation process by CCD was performed using Statistica 6.0 (Statsoft, Tulsa, USA) and Microsoft Excel 2007 for data processing.

2.5. Performance characteristics

After optimisation of the operating parameters of the ICP OES technique, the performance characteristics of the proposed procedure were established. Analytical curves were built by diluting aqueous standards. The figures of merit determined were linearity, sensitivity, limit of detection (LOD), limit of quantification (LOQ) and accuracy, the latter of which was determined by calculating the recovery percentage adding aqueous standards of Pb, Ba and Sb at $100 \mu\text{g L}^{-1}$ [23].

2.6. Application

To determine the best region for collecting GSR from the hands of the shooter, four different regions were studied: palm, back, thumb and forefinger palm (TF-palm), and thumb and forefinger back (TF-back). The GSRs were obtained from six subjects (three men and three women) who performed 1, 3 and 5 shots in triplicate for analysis of the four hand regions.

The GSR samples were collected in the following order: i) before shooting (blank); ii) 1 shot; iii) hand washing; iv) 3 shots; v) hand washing; vi) 5 shots and vii) hand washing. For the hand-washing

step, a similar procedure was performed in all cases: the shooter washed both hands with coconut soap, vinegar and, finally, deionised water. The swabs containing GSR were stored in 15 mL polypropylene tubes. After collection, 2 mL of the 10% HNO₃ solution (v/v) was added to each tube. Then, the samples were diluted to a final volume of 10 mL. The samples were subjected to extraction using an ultrasonic bath for 20 min at 25 kHz and subsequently heated for 1 h at 100 °C in a water bath before being cooled to room temperature. Finally, the swabs were removed from the tubes, and the resulting solutions were analysed by ICP OES [5,6].

3. Results and discussion

3.1. Optimisation of operating parameters

A multivariate optimisation was performed to determine the best working conditions for the three variables under study: RF power (Pow), nebuliser gas flow (Neb) and aspiration rate (Asp). The optimised parameters were to be used for the measurement of Pb, Ba and Sb in GSR by ICP OES. The 17 experiments were performed in random order, and the responses were the emission signals of all analytes (Pb/Ba/Sb). To estimate the experimental error, triplicate tests were performed at the central point. In the resulting model, the signals are not encoded. These variables were studied at five levels in the CCD (Table 1). The matrix used to optimise the three variables is presented in Table 1.

To describe the quadratic model under study, the following polynomial (Eq. (1)) was used, with three variables, x_1 (Pow), x_2 (Neb) and x_3 (Asp)], where b_0 is the mean value of the results and b_1 to b_{23} are the model coefficients to be determined:

$$\hat{y} = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3. \quad (1)$$

The coefficients of the model equation were calculated by the least squares method using the equation $\mathbf{b} = (\mathbf{X}^T\mathbf{X})^{-1}\mathbf{X}^T\mathbf{y}$, where \mathbf{X} is the contrast or design matrix, \mathbf{X}^T is its transpose matrix and vector \mathbf{y} contains the responses (intensities). Vector \mathbf{b} contains the model coefficients. To assess the quality of the fit of the model built by the optimisation of variables, an ANOVA was performed to assess the degree of fit of the model based on the variance of the replicates at the central point

Table 2

ANOVA, model validation and values of the F test analysis of variance (ANOVA).

Source of variation	Quadratic sum (QS)	Number of degrees of freedom	Quadratic mean (QM)	F _{calculated}
Pb				
Regression ^a	790.5 · 10 ⁶	9	87.84 · 10 ⁶	8.56
Residual	71.79 · 10 ⁶	7	10.26 · 10 ⁶	
Lack of fit ^b	69.62 · 10 ⁶	5	13.92 · 10 ⁶	12.9
Pure error	2.17 · 10 ⁶	2	1.08 · 10 ⁶	
Total	862.3 · 10 ⁶	16	–	
Ba				
Regression ^a	7588 · 10 ⁶	9	843.1 · 10 ⁶	10.1
Residual	583.01 · 10 ⁶	7	83.29 · 10 ⁶	
Lack of fit ^b	582.97 · 10 ⁶	5	116.6 · 10 ⁶	6.31 · 10 ³
Pure error	0.004 · 10 ⁶	2	0.02 · 10 ⁶	
Total	8171 · 10 ⁶	16	–	
Sb				
Regression ^a	104.2 · 10 ³	9	11.58 · 10 ³	26.9
Residual	3.012 · 10 ³	7	0.430 · 10 ³	
Lack of fit ^b	2.498 · 10 ³	5	0.500 · 10 ³	1.94
Pure error	0.514 · 10 ³	2	0.670 · 10 ³	
Total	107.2 · 10 ³	16	–	

^a F tabulated for regression: $F(\nu_1 = 9, \nu_2 = 7, \alpha = 0.05) = 3.68$.

^b F tabulated for Lack of fit: $F(\nu_1 = 5, \nu_2 = 2, \alpha = 0.05) = 19.3$.

[24] (Table 2). Eqs. (2), (3) and (4) represent the model fit for the three metals Pb, Ba and Sb, respectively:

$$\begin{aligned} \hat{y}_{\text{Pb}} = & 9.73 \cdot 10^3 + 1.66 \cdot 10^3 x_1 - 7.29 \cdot 10^3 x_2 - 4.30 \cdot 10^2 x_3 \\ & + 5.88 \cdot 10^2 x_1^2 + 6.83 \cdot 10^2 x_2^2 + 6.82 \cdot 10^2 x_3^2 - 1.25 \cdot 10^3 x_1 x_2 \\ & + 3.82 \cdot 10^2 x_1 x_3 + 5.37 \cdot 10^3 x_2 x_3 \end{aligned} \quad (2)$$

$$\begin{aligned} \hat{y}_{\text{Ba}} = & 8.80 \cdot 10^3 + 2.70 \cdot 10^3 x_1 - 2.19 \cdot 10^4 x_2 - 6.89 \cdot 10^2 x_3 \\ & + 2.15 \cdot 10^3 x_1^2 + 8.64 \cdot 10^3 x_2^2 + 2.36 \cdot 10^3 x_3^2 - 2.77 \cdot 10^3 x_1 x_2 \\ & + 3.48 \cdot 10^2 x_1 x_3 + 1.20 \cdot 10^3 x_2 x_3 \end{aligned} \quad (3)$$

Table 1

Levels with the respective actual values of the parameters of the multivariate optimisation study and CCD matrix for three variables.

Assay	Factors						Response (coded intensity)			
	Power (W)		Nebuliser gas flow (L·min ⁻¹)		Aspiration rate (L·min ⁻¹)		Pb	Ba	Sb	
	Real	Coded	Real	Coded	Real	Coded				
1	1200	(−1)	0.80	(−1)	0.60	(−1)	20,548.89	49,094.91	156.24	Factorial design
2	1400	(+1)	0.80	(−1)	0.60	(−1)	24,825.92	58,769.57	152.13	
3	1200	(−1)	1.20	(+1)	0.60	(−1)	2962.50	0	5.23	
4	1400	(+1)	1.20	(+1)	0.60	(−1)	4878.83	0	0	
5	1200	(−1)	0.80	(−1)	1.00	(+1)	15,947.23	42,885.45	220.98	
6	1400	(+1)	0.80	(−1)	1.00	(+1)	24,401.69	55,344.53	109.54	
7	1200	(−1)	1.20	(+1)	1.00	(+1)	3155.47	0	0	
8	1400	(+1)	1.20	(+1)	1.00	(+1)	3954.19	0	0	
9	1132	(−1.68)	1.00	(0)	0.80	(0)	7963.11	5048.66	38.36	Axial points
10	1468	(+1.68)	1.00	(0)	0.80	(0)	12,276.87	13,829.56	34.25	
11	1300	(0)	0.67	(−1.68)	0.80	(0)	18,928.41	55,513.65	270.89	
12	1300	(0)	1.34	(+1.68)	0.80	(0)	1845.94	0	0	
13	1300	(0)	1.00	(0)	0.46	(−1.68)	10,415.83	9967.22	54.27	Replicates' central point
14	1300	(0)	1.00	(0)	1.14	(+1.68)	10,350.62	10,102.90	39.67	
15	1300	(0)	1.00	(0)	0.80	(0)	10,506.98	9425.59	39.53	
16	1300	(0)	1.00	(0)	0.80	(0)	8681.89	9585.19	71.41	
17	1300	(0)	1.00	(0)	0.80	(0)	10,462.39	9314.78	52.48	

$$\hat{y}_{Sb} = \frac{5.40}{(\pm 2.83)} \cdot 10 - \frac{9.35}{(\pm 13.3)} x_1 - \frac{7.98}{(\pm 13.3)} \cdot 10x_2 - \frac{0.56}{(\pm 13.3)} x_3 - \frac{5.00}{(\pm 14.6)} x_1^2 + \frac{3.01}{(\pm 1.46)} \cdot 10x_2^2 - \frac{1.22}{(\pm 14.6)} x_3^2 + \frac{1.38}{(\pm 1.73)} \cdot 10x_1x_2 - \frac{1.28}{(\pm 1.73)} \cdot 10x_1x_3 - \frac{3.42}{(\pm 17.3)} x_2x_3 \quad (4)$$

The values in parentheses are the confidence interval of the coefficients. Their significance was assessed by Student's t-test, as shown in Fig. 1S (see Supplementary material). The uncertainty bars that include zero indicate that the corresponding coefficient is not significant at the 95% confidence level.

The results presented in Fig. 1S show that nebuliser gas flow (Neb) is the only important parameter in the optimisation of the experimental conditions for the determination of Pb, Ba and Sb. For Pb, only the linear term is significant, whereas for Ba and Sb the linear and quadratic terms are significant. This can be explained by the fact that the GSR samples are soluble in aqueous medium and thus do not interfere in the excitation and atomisation processes of the analytes. The interactions between parameters were not significant in any model.

The good fit of the models to the experimental data can be observed in Fig. 2S. The models have correlation coefficients of 0.9167, 0.9287 and 0.9719 for Pb, Ba and Sb, respectively.

The results of the ANOVA F-test are shown in Table 2. By examining the F test values for the degree of fit, it can be observed that the models for Pb and Sb do not exhibit lack of fit because their $F_{\text{calculated}}$ values are lower than the critical value of 19.3 at the 95% confidence level. In addition, their regressions are significant at the same confidence level because their $F_{\text{calculated}}$ values are higher than the F_{critical} value (3.68). The model for Ba presents an optimal value for coefficient of determination (R^2); however, it lacks fit because $F_{\text{calculated}} > F_{\text{critical}}$. Hence, for Ba, it would not be valid to perform a regression significance test. A new ANOVA F-test was performed without the significant coefficients for the Pb and Sb models (Table 3). Now, similar to Ba, the model for Pb without non-significant coefficients showed lack of fit, $F_{\text{calculated}} > F_{\text{critical}}$ ($4.4 > 3.49$) whereas for the Sb model, better results are obtained in relation to those with all coefficients, $F_{\text{calculated}} < F_{\text{critical}}$ ($0.02 < 3.89$). Also, the ratio of mean square regression by the residual is notably higher than the F_{critical} value ($94 > 3.74$), showing thus that the regression is highly significant.

Only the linear factor of the nebuliser gas flow variable (x_2) was significant in the model for determining Pb. The Ba model has lacked fit and thus cannot be used to obtain other information about this model. However, the linear and quadratic factors of the nebuliser gas flow variable were significant in the Sb model. To establish a single

optimal experimental condition for the simultaneous measurement of the three elements (Pb, Ba and Sb), the following analysis was performed:

- Pb model: according to Eq. (2), the signal intensity increases with the reduction of the nebuliser gas flow, due to the negative coefficient ($b_2 = -7.29 \cdot 10^3$) of the fitted model.
- Sb model: only the linear and quadratic terms are significant for this model (Fig. 1). Thus, the optimal experimental conditions can be determined by maximising Eq. (4) (using only terms with significant coefficients) relative to variable x_2 (nebuliser gas flow):

$$\frac{\partial \hat{y}_{Sb}}{\partial x_2} = -7.98 \cdot 10 + 6.02x_2 = 0 \quad (5)$$

$$x_2 \approx 1.3.$$

Considering that the sensitivity for determination of Sb by ICP OES is lower than for the other elements and that the Ba model also has a value of x_2 (using the same optimisation process for Sb) close to 1.3, the optimal nebuliser gas flow for analysis of the three elements used was 1.0, equivalent to 1.2 L min^{-1} . The power and aspiration rates were set to 1300 W and 1.0 mL min^{-1} , respectively.

3.2. Determination of performance characteristics

After determining the optimal operating conditions, calibration curves were prepared with five points at the following concentrations: 0, 100, 300, 500 and $1000 \mu\text{g} \cdot \text{L}^{-1}$ for Pb, Ba and Sb. To verify the presence of possible matrix interferences, an internal standard (IS) was used. The IS tested were yttrium (Y), gallium (Ga) and scandium (Sc).

From the calibration curves obtained, the performance characteristics were established for the proposed method: linearity, LOD, LOQ, and recovery percentage, for Pb, Ba and Sb (Table 4), with and without IS addition. The values are demonstrated in $\mu\text{g L}^{-1}$. The LOD and LOQ are parameters related to the sensitivity of the method; the lower the LOD and LOQ, the higher the sensitivity. The LOD was calculated as 3 times the standard deviation (s) of 10 blank sample readings, divided by the calibration curve slope (a), $3s/a$, and the LOQ was calculated as 10 times the standard deviation (s) of 10 sample blank readings, divided by the calibration curve slope, $10s/a$. The linearity was evaluated by the R^2 of the curve. The recovery percentage was calculated to check the accuracy of the procedure; the recommended recovery is 80–120% [25]. In this study, recoveries ranged from 92% to 120% without the use of IS, indicating good accuracy.

Tests performed on real GSR samples showed that the proposed conditions are in accordance with expectations, and it is possible to detect concentrations of Pb, Ba and Sb above the LOD (Table 5) for 3 to 5 shots without using ISs, even if the shooter's hands are washed after the shots. This indicates that the proposed method can be used to identify gunshot residues accurately. Thus, the results indicate that there is no need to use ISs, considering the strong trend toward linearity and good recovery for tests conducted without ISs.

3.3. Applications

To check the efficiency of the proposed optimisation, some simulated cases were analysed to detect gunshot residues from a .38 revolver. A study to determine the best shooter hand region for collection (palm, back, TF-palm, and TF-back) and the effect of hand washing after the shots was performed, and the data are presented in Fig. 1(a–d) [5,26]. When the sensitivity was analysed as a function of the hand region, the highest concentrations of the three elements (Pb, Ba and Sb) were found mainly in the palm, TF-palm and TF-back regions. However, when the hand washing effect was analysed, the three elements were detected only in the TF-palm and TF-back regions after three and five

Table 3

ANOVA, model validation and values of the F test analysis of variance (ANOVA) without non-significant coefficients.

Source of variation	Quadratic sum (QS)	Number of degrees of freedom	Quadratic mean (QM)	$F_{\text{calculated}}$
Pb				
Regression ^a	$7.25 \cdot 10^8$	1	$7.25 \cdot 10^8$	79
Residual	$1.37 \cdot 10^8$	15	$9.14 \cdot 10^6$	
Lack of fit ^b	$7.19 \cdot 10^7$	3	$2.40 \cdot 10^7$	4.4
Pure error	$6.53 \cdot 10^7$	12	$5.44 \cdot 10^6$	
Total	$8.62 \cdot 10^8$	16	–	
Sb				
Regression ^c	$9.98 \cdot 10^4$	2	$4.99 \cdot 10^4$	94
Residual	$7.41 \cdot 10^3$	14	$5.29 \cdot 10^2$	
Lack of fit ^d	24	2	12	0.02
Pure error	$7.39 \cdot 10^3$	12	$6.16 \cdot 10^2$	
Total	$1.07 \cdot 10^5$	16	–	

^a F tabulated for regression: $F(v_1 = 1, v_2 = 15, \alpha = 0.05) = 4.54$.

^b F tabulated for Lack of fit: $F(v_1 = 3, v_2 = 12, \alpha = 0.05) = 3.49$.

^c F tabulated for regression: $F(v_1 = 2, v_2 = 14, \alpha = 0.05) = 3.74$.

^d F tabulated for Lack of fit: $F(v_1 = 2, v_2 = 12, \alpha = 0.05) = 3.89$.

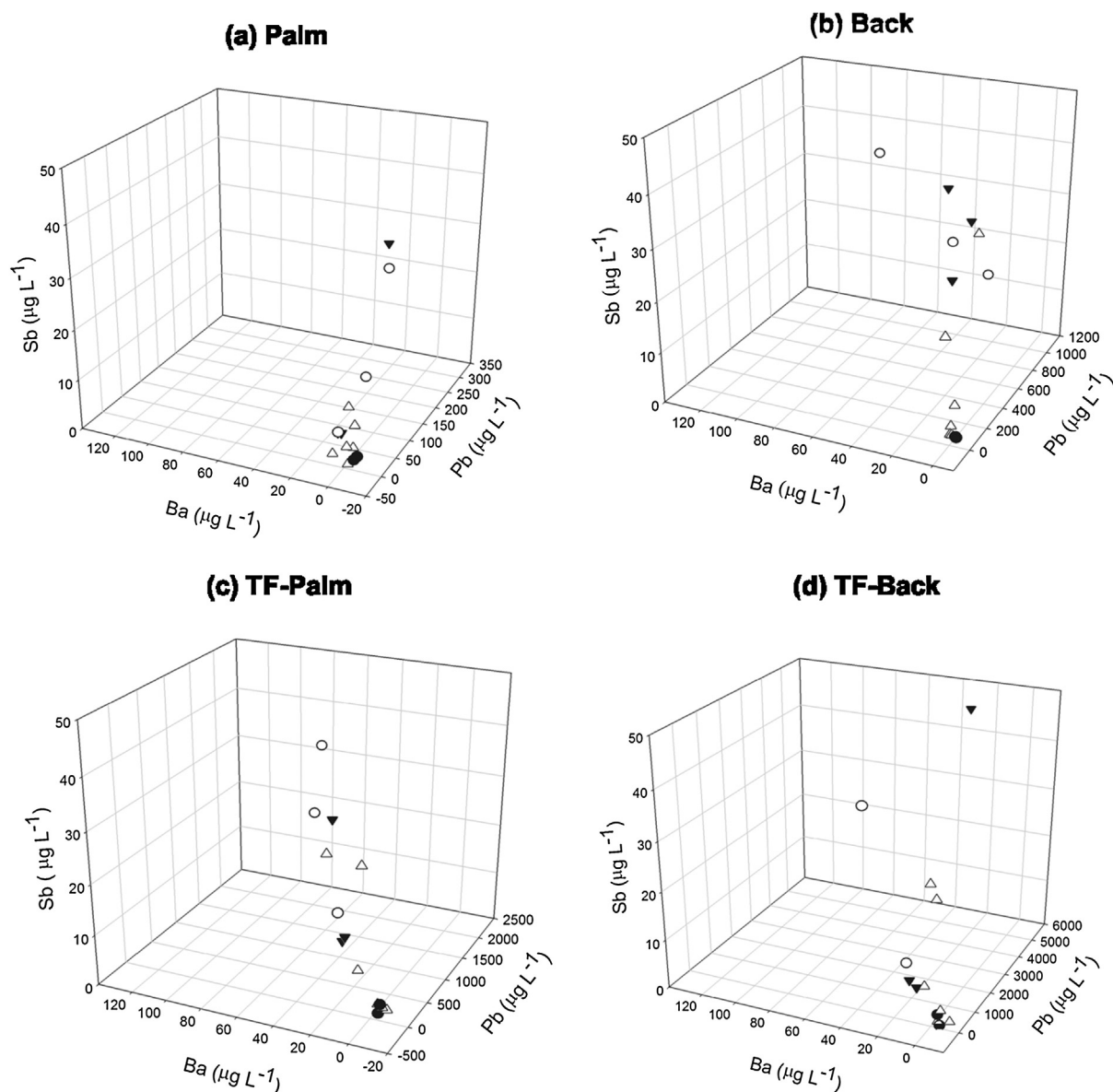


Fig. 1. 3D graph of Pb, Ba and Sb as a function of collection region: (a) palm; (b) back; (c) thumb and forefinger palm; and (d) Thumb and forefinger back. The symbols correspond to (●) blank; (○) man; (▼) woman; and (▲) hand washing. [5].

shots. These two regions are the most sensitive, generating more representative results as a function of the number of shots and hand washing. This difference may be explained by the presence of a larger number of

folids in these hand regions, which are able to store greater amounts of GSR, even after washing. Therefore, the results found are satisfactory because most murder cases involving firearms involve an average of 10 to 12 shots on the victim, and cases in which the victim is shot only once are rare [27]. This result indicates that the proposed method is sensitive for most cases because it can detect GSR produced by 3 or more shots, even if the shooter washes the hand [5].

Table 4
Figures of merit for Pb, Ba and Sb in GSR.

Element	IS	Performance characteristics			
		Linearity	LOD ($\mu\text{g L}^{-1}$)	LOQ ($\mu\text{g L}^{-1}$)	Recovery %
Pb	–	0.9999	1.49	4.97	119.69
	Y	0.9966	3.25	10.83	227.60
	Ga	0.9989	7.44	24.82	213.11
	Sc	0.9999	8.23	27.44	157.53
Ba	–	0.9999	0.15	0.50	96.73
	Y	0.9974	0.80	2.66	124.70
	Ga	0.9948	0.69	2.30	121.74
	Sc	0.9998	0.66	2.20	137.57
Sb	–	0.9999	4.79	15.97	92.97
	Y	0.9973	9.81	32.70	83.86
	Ga	0.9988	5.33	17.77	113.36
	Sc	0.9997	5.54	18.46	120.06

Table 5
Pb, Ba and Sb concentrations for 3 and 5 shots before and after hand washing. The values in parentheses are the standard deviations based on three measurements.

Sample	Metals		
	Pb ($\mu\text{g L}^{-1}$)	Ba ($\mu\text{g L}^{-1}$)	Sb ($\mu\text{g L}^{-1}$)
3 shots	1047.11 (3.11) ^a	41.93 (0.79)	20.49 (1.02)
3 shots and hand washing	27.82 (1.45)	4.56 (2.01)	20.00 (3.01)
5 shots	5183.18 (0.02)	92.12 (0.45)	33.41 (0.90)
5 shots and hand washing	626.95 (0.96)	14.61 (0.66)	28.89 (0.02)

^a The value between parentheses corresponds to standard deviations based on three measurements.

4. Conclusion

Multivariate optimisation with a central composite design was efficient for determining the best operating conditions for the ICP OES technique, as used in the analysis of Pb, Ba and Sb in GSR samples. A good linear relationship between the experimental and predicted values for the model was obtained ($R^2_{Pb} = 0.9167$; $R^2_{Ba} = 0.9287$; and $R^2_{Sb} = 0.9719$). Despite the good correlation coefficient, the Ba model lacks fit and therefore should be discarded. Using only the significant coefficients, an improvement was observed for the model for Sb. The nebuliser gas flow is the most important parameter in the optimisation of the signal intensities, and the interactions between the variables were not significant. Thus, the following values were determined for RF power, nebuliser gas flow and aspiration rate as compromise conditions: 1300 W; 1.2 L min⁻¹; and 1.0 mL min⁻¹, respectively. The values found for the limits of detection and quantification were as follows: 1.49 and 4.97 µg L⁻¹ for Pb; 0.15 and 0.50 µg L⁻¹ for Ba; and 4.79 and 15.97 µg L⁻¹ for Sb. The analysis of the figures of merit (sensitivity, linearity, limit of detection, limit of quantification and accuracy) indicated that the experimental conditions obtained by the experimental design are satisfactory. The addition of an internal standard in the analysis is not required because the optimised conditions provide good sensitivity and accuracy.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.microc.2015.01.003>.

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